

WHAT IS CLAIMED IS:

1 1. A method of reassembling polynucleotides involved in
2 transcription, the method comprising,
3 providing a plurality of random polynucleotide segments from one or more
4 transcriptional regulatory progenitor polynucleotides;
5 assembling the plurality of segments in a random fashion, thereby forming
6 a plurality of reassembled polynucleotide; and
7 selecting a reassembled polynucleotide with a different transcriptional
8 regulatory activity than the progenitor polynucleotides.

1 2. The method of claim 1, wherein the segments are from 5 bp to
2 5,000 bp long.

1 3. The method of claim 1, wherein the segments are less than 50 base
2 pairs.

1 4. The method of claim 1, wherein the segments are greater than 49
2 base pairs.

1 5. The method of claim 1, wherein the assembling step comprises
2 ligating the segments.

1 6. The method of claim 5, wherein the ligating step is performed by
2 with a DNA ligase or a topoisomerase.

1 7. The method of claim 1, wherein the plurality of random segments
2 comprises segments from at least two distinct promoter or enhancer polynucleotides.

1 8. The method of claim 1, wherein the plurality of random
2 polynucleotide segments are obtained by random cleavage of one or more transcriptional
3 regulatory progenitor polynucleotides.

1 9. The method of claim 1, wherein the plurality of random
2 polynucleotide segments are obtained by random amplification of one or more part of one
3 or more transcriptional regulatory progenitor polynucleotides.

- 1 10. The method of claim 1, wherein the reassembled polynucleotide
2 comprises a promoter.
- 1 11. The method of claim 1, wherein the reassembled polynucleotide
2 comprises an enhancer.
- 1 12. The method of claim 1, wherein the selection step comprises
2 selecting a reassembled polynucleotide with increased transcriptional activity relative to
3 the transcriptional activity of a progenitor polynucleotide.
- 1 13. The method of claim 1, wherein the selection step comprises
2 selecting a reassembled polynucleotide with decreased transcriptional activity relative to
3 the transcriptional activity of a progenitor polynucleotide.
- 1 14. The method of claim 1, wherein the selection step comprises
2 selecting a reassembled polynucleotide with significant transcriptional activity in at least
3 one cell or tissue type where the progenitor polynucleotide lacks activity.
- 1 15. The method of claim 1, wherein the selection step comprises
2 selecting a reassembled polynucleotide without significant transcriptional activity in at
3 least one cell or tissue type where the progenitor polynucleotide has activity.
- 1 16. The method of claim 1, wherein the selection step comprises
2 selecting a reassembled polynucleotide with transcriptional activity that is activated in
3 response to biotic or abiotic stimuli.
- 1 17. The method of claim 1, where the segments are formed by nicking
2 and subsequent end-repair of DNA that is altered by radiation, oxidation, or a chemical
3 agent.
- 1 18. The method of claim 1, wherein the selection step comprises
2 selecting a reassembled polynucleotide with transcriptional activity at a different
3 developmental stage of an organism relative to the transcriptional activity of a progenitor
4 polynucleotide.
- 1 19. The method of claim 1, wherein the segments are formed by
2 cleaving one or more progenitor polynucleotides with a restriction endonuclease.

- 1 20. The method of claim 1, wherein the segments are formed by
2 cleaving one or more progenitor polynucleotides with DNaseI.
- 1 21. The method of claim 1, wherein the segments are formed by
2 cleaving one or more progenitor polynucleotides mechanically.
- 1 22. The method of claim 1, wherein the segments are formed in a
2 thermocyclic amplification reaction.
- 1 23. The method of claim 22, wherein the thermocyclic reaction is a
2 polymerase chain reaction.
- 1 24. The method of claim 23, wherein the polymerase chain reaction is
2 a mutagenic polymerase chain reaction.
- 1 25. The method of claim 1, wherein the selection step is performed by
2 ligating the reassembled polynucleotide to a reporter gene and measuring reporter gene
3 activity.
- 1 26. The method of claim 1, wherein the plurality of segments further
2 comprises oligonucleotides.
- 1 27. The method of claim 26, wherein the oligonucleotide sequence
2 corresponds to a transcription factor binding site.
- 1 28. The method of claim 26, wherein the nucleotide sequence of the
2 oligonucleotides are not from a transcriptional regulatory polynucleotide.
- 1 29. The method of claim 1, wherein the reassembled polynucleotide is
2 shorter than the progenitor polynucleotide.
- 1 30. The method of claim 1, wherein the reassembled polynucleotide is
2 longer than the progenitor polynucleotide.
- 1 31. The method of claim 1, wherein the progenitor polynucleotides
2 comprise allelic variants of a transcriptional regulator polynucleotide.

1 32. The method of claim 1, wherein the progenitor polynucleotides
2 comprise plant transcriptional regulatory polynucleotides.

1 33. The method of claim 1, wherein the progenitor polynucleotides
2 comprise yeast transcriptional regulatory polynucleotides.

1 34. The method of claim 1, wherein the progenitor polynucleotides
2 comprise fungal transcriptional regulatory polynucleotides.

1 35. The method of claim 1, wherein the progenitor polynucleotides
2 comprise mammalian transcriptional regulatory polynucleotides.

1 36. The method of claim 1, wherein the progenitor polynucleotides
2 comprise viral transcriptional regulatory polynucleotides.

1 37. The method of claim 1, wherein the progenitor polynucleotides
2 comprise bacterial transcriptional regulatory polynucleotides.

1 38. The method of claim 1, wherein the progenitor polynucleotides
2 consist of one transcriptional regulatory polynucleotide.

1 39. The method of claim 1, wherein the transcriptional regulatory
2 progenitor polynucleotides comprise more than one transcriptional regulatory
3 polynucleotide.

1 40. The method of claim 1, wherein the transcriptional regulatory
2 progenitor polynucleotides are less than 70% identical.

1 41. The method of claim 1, wherein the progenitor polynucleotides are
2 less than 50% identical.

1 42. The method of claim 1, wherein the progenitor polynucleotides do
2 not hybridize to each other following at least one wash in 0.2X SSC at 55° C for 20
3 minutes.

1 43. The method of claim 1, wherein the polynucleotide segments are
2 single stranded.

1 44. The method of claim 1, wherein the polynucleotide segments are
2 double-stranded.

1 45. The method of claim 44, wherein the double-stranded segments
2 have at least one overhanging single-stranded end.

1 46. The method of claim 45, wherein the overhanging single-stranded
2 end comprises fewer than 10 base pairs.

1 47. The method of claim 1, wherein the assembling step does not
2 comprise a polymerase.

1 48. A reassembled polynucleotide of claim 1.